

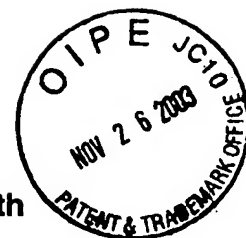
REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

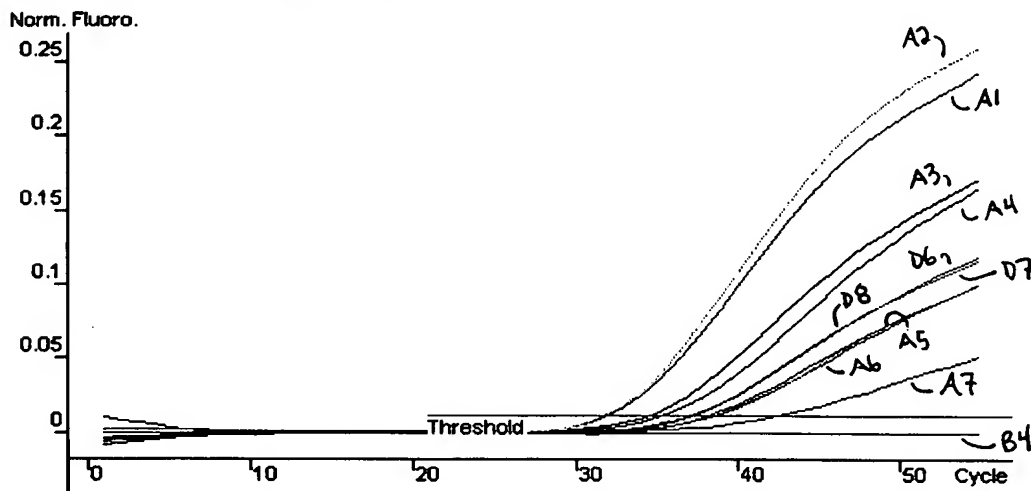
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

Docket No.: 546322000100



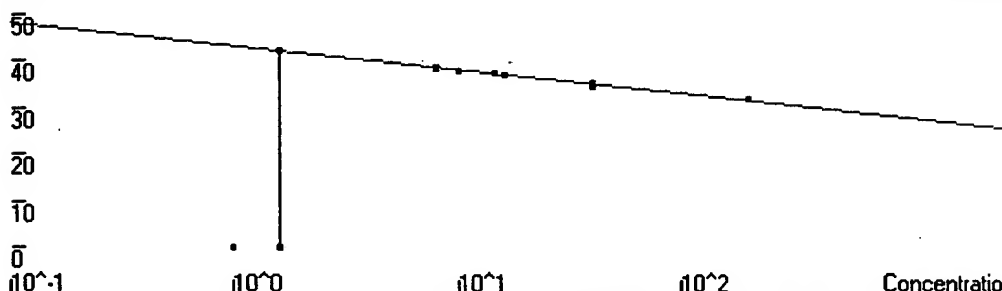
Amplification plots and Quantitation data for Human BRN2 (Duplexed with Human GAPDH Figur 1b)



Standard Curve

60 CT

R = 0.99625



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	174.9	12.54%	31.94	0.07
A2		dT SS RNA 200ng	Standard	200.0	187.5	6.25%	31.79	0.07
A3		dT SS RNA 40ng	Standard	40.0	54.2	35.54%	34.47	0.43
A4		dT SS RNA 40ng	Standard	40.0	36.4	8.98%	35.33	0.43
A5		dT SS RNA 8ng	Standard	8.0	7.9	1.70%	38.64	0.13
A6		dT SS RNA 8ng	Standard	8.0	8.9	10.88%	38.38	0.13
A7		dT SS RNA 1.6ng	Standard	1.6	1.5	9.30%	42.29	21.15
A8		dT SS RNA 1.6ng	Standard	1.6				21.15
B4		RTminus MM96L 2.1.1, NRO 10 ⁶ nuclei	Sample					
D6		RT plus MM96L 2.1.1, NRO 10 ⁶ nuclei	Sample		16.3		37.07	
D7		RT plus MM96L 2.1.1, NRO 10 ⁶ nuclei	Sample		14.5		37.32	
D8		RT plus MM96L 2.1.1, NRO 10 ⁶ nuclei	Sample		10.1		38.11	

Figure 1a

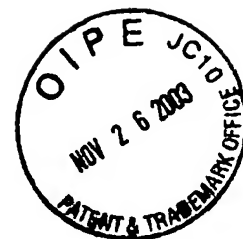
REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

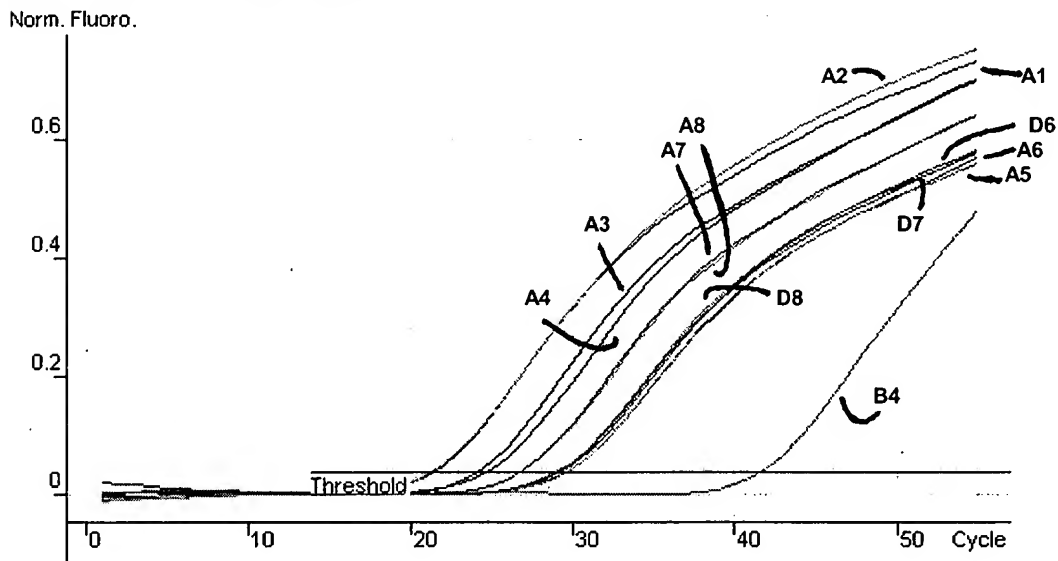
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

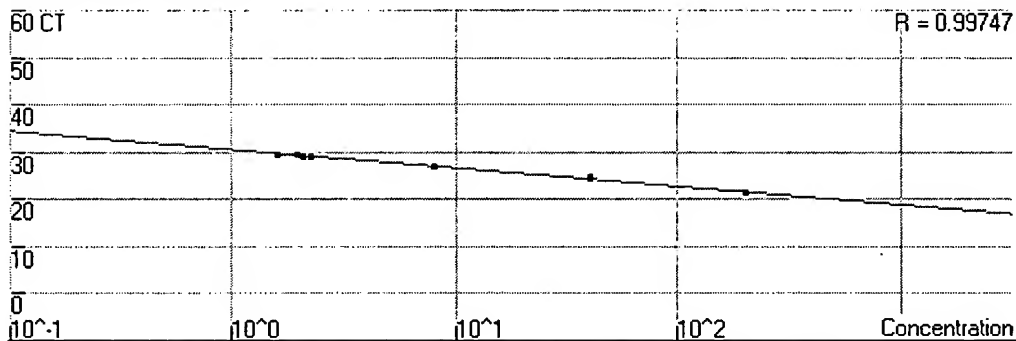
Docket No.: 546322000100



Amplification plots and Quantitation data for Human GAPDH (Duplexed with Human BRN2 Figure 1a)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	210.2	5.10%	21.37	0.04
A2		dT SS RNA 200ng	Standard	200.0	220.4	10.18%	21.29	0.04
A3		dT SS RNA 40ng	Standard	40.0	40.3	0.78%	24.17	0.29
A4		dT SS RNA 40ng	Standard	40.0	28.8	28.00%	24.74	0.29
A5		dT SS RNA 8ng	Standard	8.0	8.4	5.57%	26.82	0.01
A6		dT SS RNA 8ng	Standard	8.0	8.5	6.82%	26.8	0.01
A7		dT SS RNA 1.6ng	Standard	1.6	1.6	0.55%	29.65	0.06
A8		dT SS RNA 1.6ng	Standard	1.6	1.7	6.12%	29.54	0.06
B4		RTminus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		0.0		41.63	
D6		RT plus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		2.3		29.05	
D7		RT plus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		2.0		29.29	
D8		RT plus MM96L 2.1.1 NRO 10 ⁶ nuclei	Sample		2.1		29.18	

Figure 1b

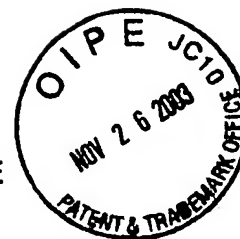
REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

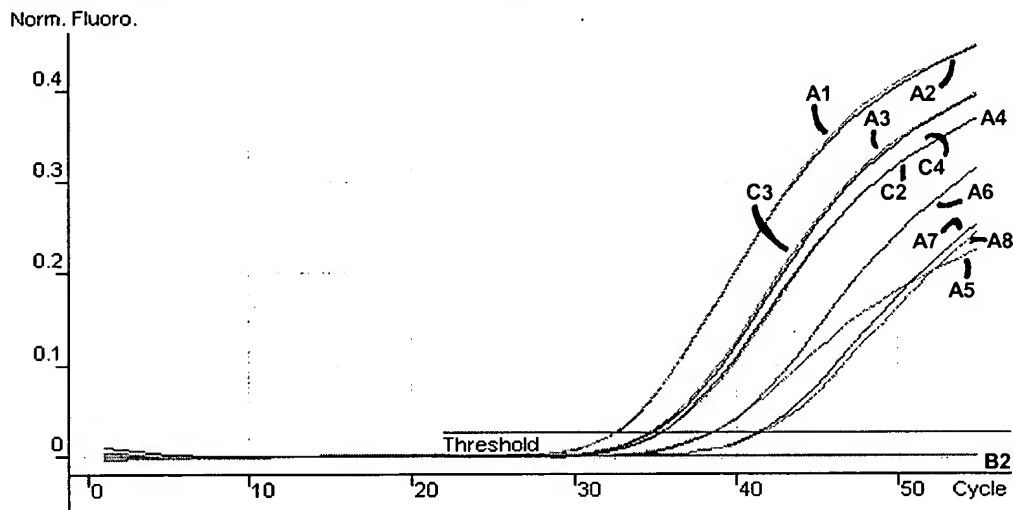
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

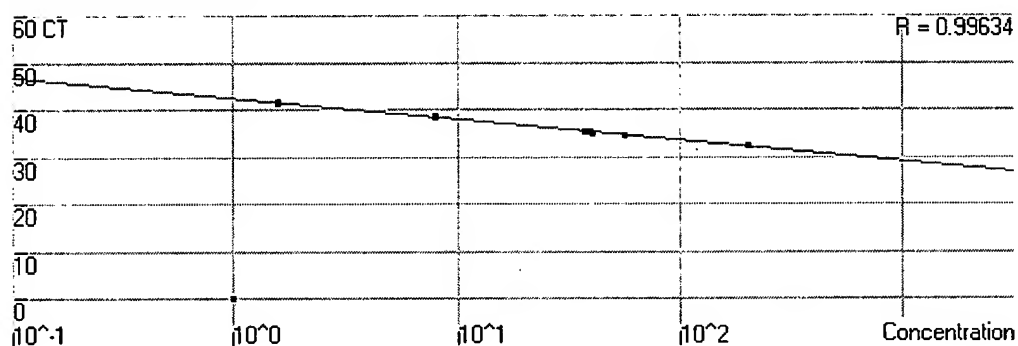
Docket No.: 546322000100



Amplification plots and Quantitation data for Murine B16 TYROSINASE (Duplexed with Murine GAPDH Figure 2b)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT SS RNA 200ng	Standard	200.0	166.8	16.60%	32.52	0.05
A2		dT SS RNA 200ng	Standard	200.0	175.7	12.16%	32.42	0.05
A3		dT SS RNA 40ng	Standard	40.0	52.2	30.55%	34.76	0.06
A4		dT SS RNA 40ng	Standard	40.0	49.1	22.67%	34.88	0.06
A5		dT SS RNA 8ng	Standard	8.0	7.9	1.12%	38.4	0.10
A6		dT SS RNA 8ng	Standard	8.0	7.1	10.86%	38.6	0.10
A7		dT SS RNA 1.6ng	Standard	1.6	1.7	6.56%	41.36	0.16
A8		dT SS RNA 1.6ng	Standard	1.6	1.5	9.26%	41.67	0.16
B2		RTminus B16 TYR Parental NRO 10 ⁶ nuclei	Sample					
C2		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		37.1		35.42	
C3		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		56.2		34.62	
C4		RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		39.1		35.32	

Figure 2a

REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

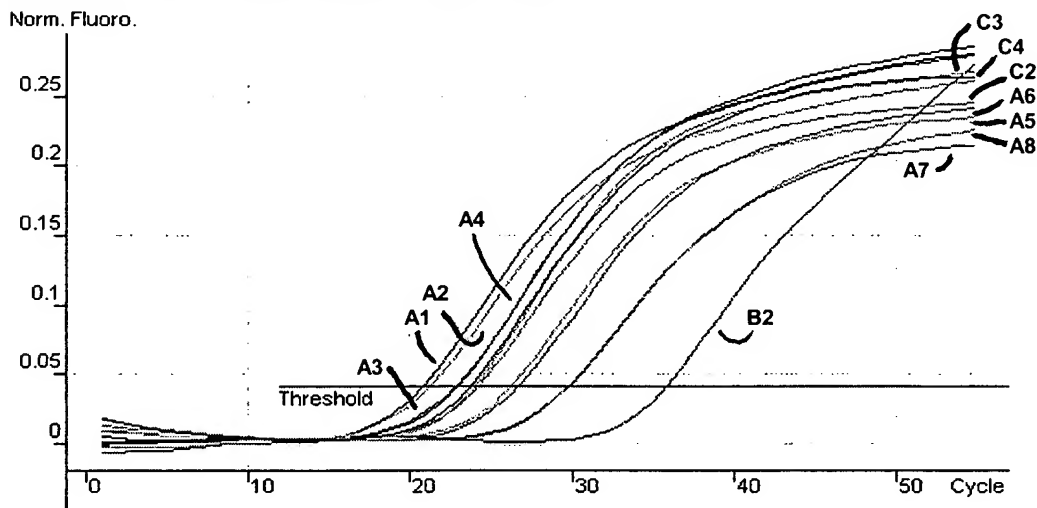
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

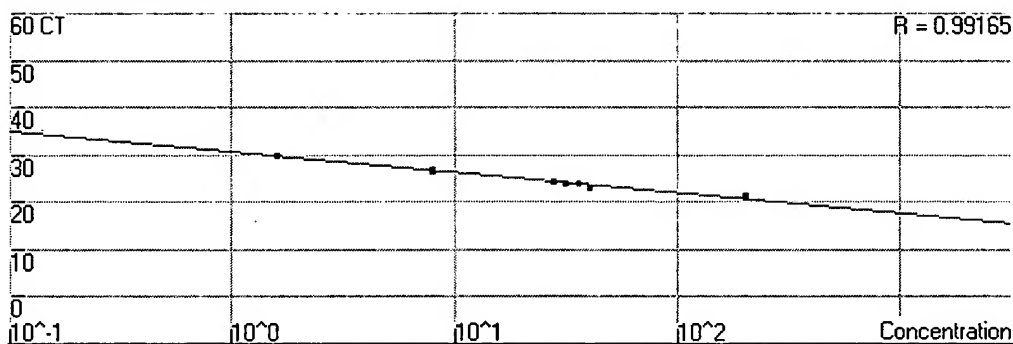
Docket No.: 546322000100



Amplification plots and Quantitation data for GAPDH (Duplexed with Murine B16 TYROSINASE Figure 2a)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1	■	dT SS RNA 200ng	Standard	200.0	168.4	15.81%	20.91	0.20
A2	■	dT SS RNA 200ng	Standard	200.0	136.9	31.55%	21.3	0.20
A3	■	dT SS RNA 40ng	Standard	40.0	53.5	33.75%	23.07	0.07
A4	■	dT SS RNA 40ng	Standard	40.0	57.6	44.07%	22.93	0.07
A5	■	dT SS RNA 8ng	Standard	8.0	9.2	14.78%	26.39	0.19
A6	■	dT SS RNA 8ng	Standard	8.0	7.5	6.19%	26.77	0.19
A7	■	dT SS RNA 1.6ng	Standard	1.6	1.5	7.08%	29.82	0.03
A8	■	dT SS RNA 1.6ng	Standard	1.6	1.4	9.99%	29.88	0.03
B2	■	RTminus B16 TYR Parental NRO 10 ⁶ nuclei	Sample		0.1		35.94	
C2	■	RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		27.8		24.3	
C3	■	RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		31.1		24.09	
C4	■	RT plus B16 TYR parental NRO 10 ⁶ nuclei	Sample		35.9		23.82	

Figure 2b

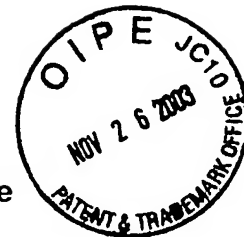
REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

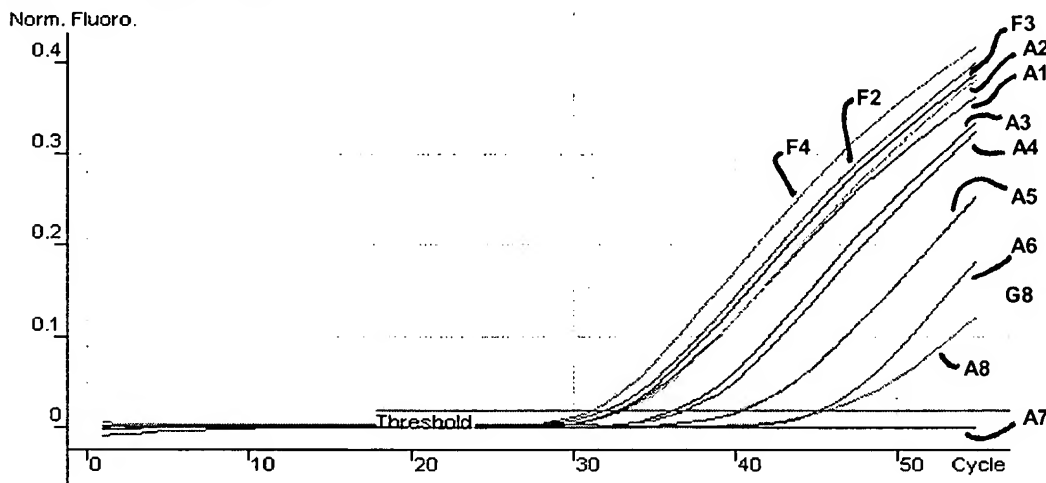
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

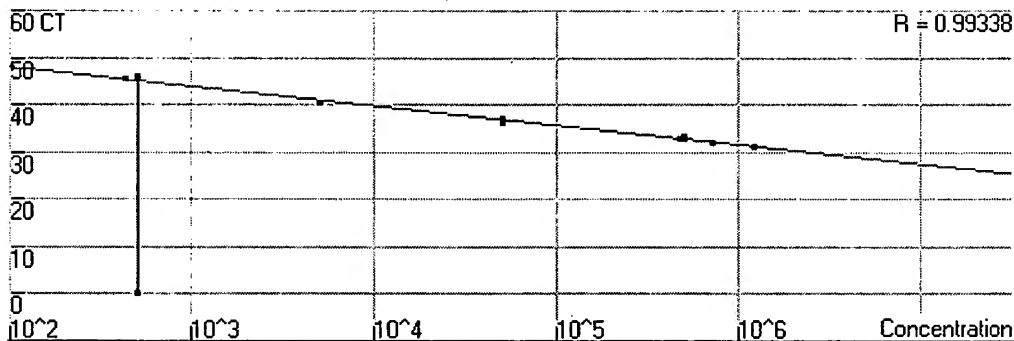
Docket No.: 546322000100



Amplification plots and Quantitation data for EGFP (Duplexed with Murine GAPDH Figure 3b)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT/SS RNA 500ng	Standard	500,000	438,882	12.22%	32.97	0.12
A2		dT/SS RNA 500ng	Standard	500,000	381,732	23.65%	33.22	0.12
A3		dT/SS RNA 50ng	Standard	50,000	65,817	31.63%	36.37	0.33
A4		dT/SS RNA 50ng	Standard	50,000	45,539	8.92%	37.03	0.33
A5		dT/SS RNA 5ng	Standard	5,000	7,062	41.23%	40.37	0.07
A6		dT/SS RNA 5ng	Standard	5,000	6,531	30.62%	40.51	0.07
A7		dT/SS RNA 0.5ng	Standard	500				22.91
A8		dT/SS RNA 0.5ng	Standard	500	337	32.53%	45.82	22.91
F2		RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		717,169		32.09	
F3		RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		477,201		32.82	
F4		RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		1,198,365		31.17	
G8		RT minus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		429		45.39	

Figure 3a

REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
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Inventor: Robert N. RICE et al.

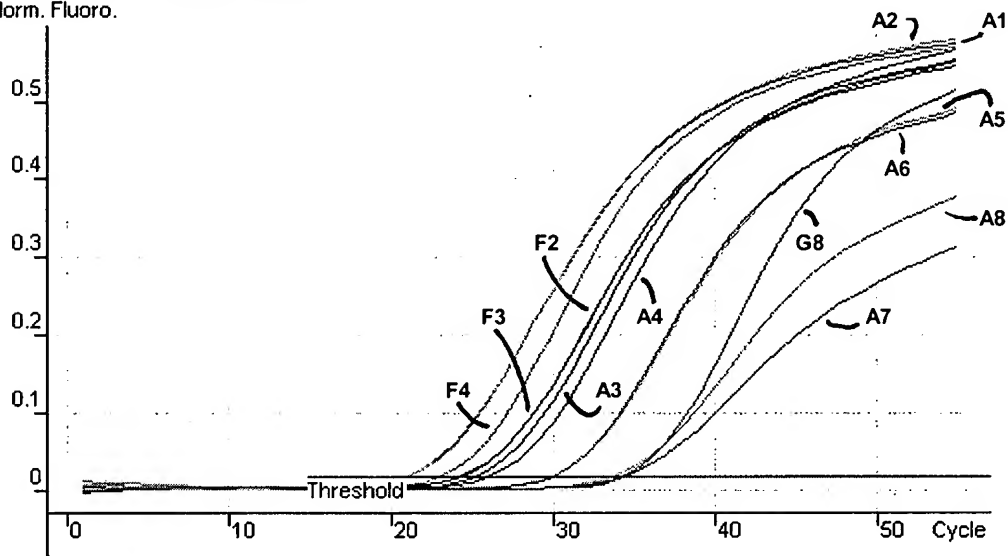
Application No.: 10/081,646

Docket No.: 546322000100

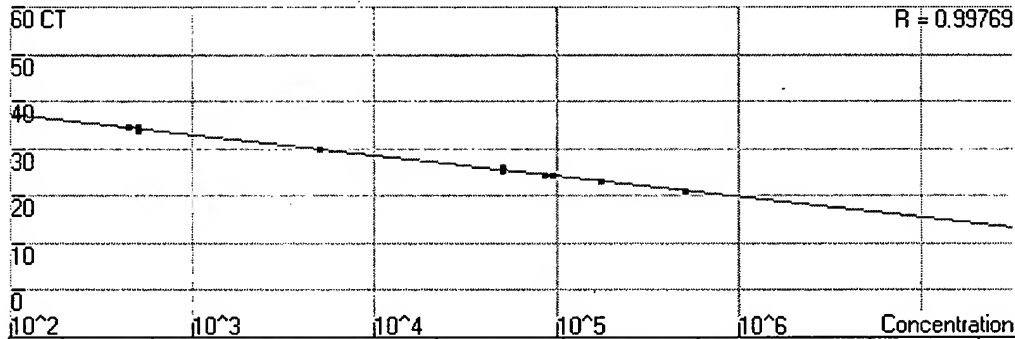


Amplification plots and Quantitation data for Murine GAPDH (Duplexed with EGFP Figure 3a)

Norm. Fluoro.



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT/SS RNA 500ng	Standard	500,000	554,947	10.99%	20.89	0.09
A2		dT/SS RNA 500ng	Standard	500,000	504,712	0.94%	21.07	0.09
A3		dT/SS RNA 50ng	Standard	50,000	57,828	15.66%	25.18	0.44
A4		dT/SS RNA 50ng	Standard	50,000	36,174	27.65%	26.07	0.44
A5		dT/SS RNA 5ng	Standard	5,000	4,829	3.42%	29.89	0.02
A6		dT/SS RNA 5ng	Standard	5,000	4,728	5.43%	29.93	0.02
A7		dT/SS RNA 0.5ng	Standard	500	436	12.71%	34.45	0.41
A8		dT/SS RNA 0.5ng	Standard	500	669	33.79%	33.64	0.41
F2		RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		85,418		24.44	
F3		RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		93,426		24.27	
F4		RT plus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		172,197		23.11	
G8		RTminus B16 EGFP #12 NRO 10 ⁶ nuclei	Sample		443		34.42	

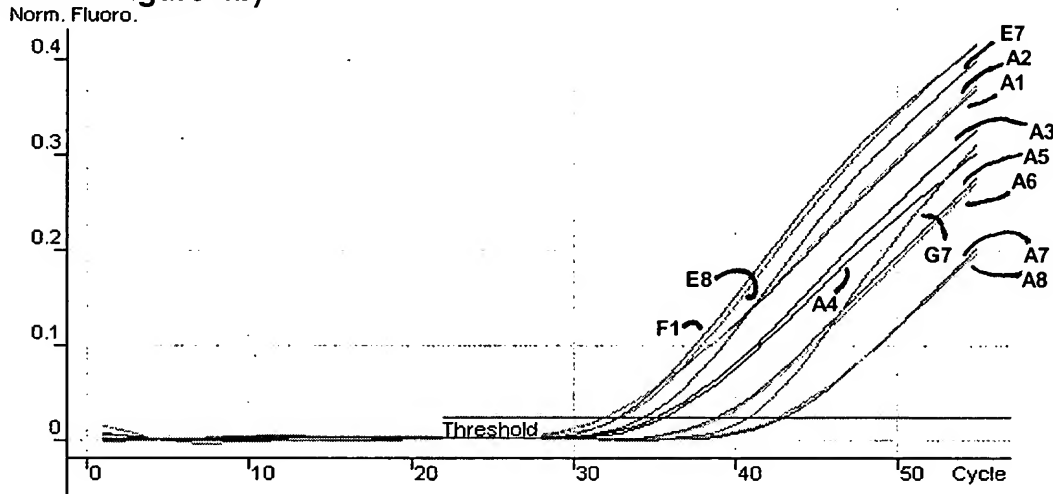
Figure 3b

REPLACEMENT SHEET

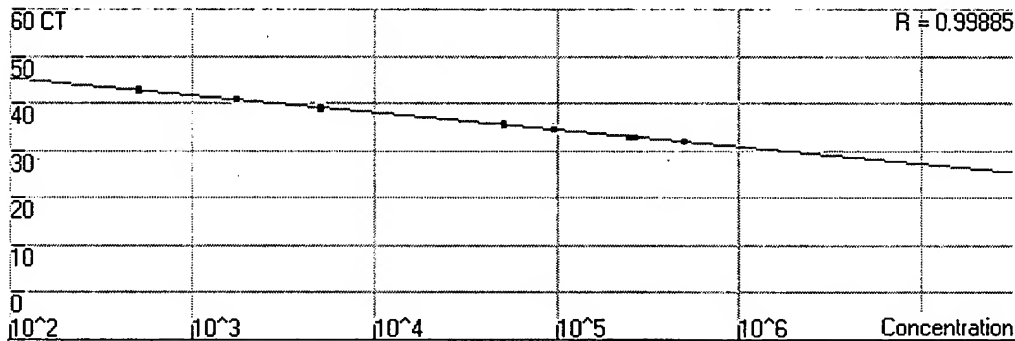
Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)
Inventor: Robert N. RICE et al.
Application No.: 10/081,646
Docket No.: 546322000100



Amplification plots and Quantitation data for EGFP (Duplexed with Human GAPDH Figure 4b)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT/SS RNA 500ng	Standard	500,000	475,544	4.89%	31.94	0.01
A2		dT/SS RNA 500ng	Standard	500,000	481,621	3.68%	31.92	0.01
A3		dT/SS RNA 50ng	Standard	50,000	60,025	20.05%	35.2	0.26
A4		dT/SS RNA 50ng	Standard	50,000	43,148	13.70%	35.72	0.26
A5		dT/SS RNA 5ng	Standard	5,000	4,889	2.22%	39.15	0.15
A6		dT/SS RNA 5ng	Standard	5,000	5,877	17.55%	38.86	0.15
A7		dT/SS RNA 0.5ng	Standard	500	422	15.67%	43.01	0.20
A8		dT/SS RNA 0.5ng	Standard	500	544	8.71%	42.61	0.20
E7		RT Plus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		94,810		34.48	
E8		RT Plus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		244,164		32.99	
F1		RT Plus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		265,171		32.86	
G7		RT minus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		1,759		40.76	

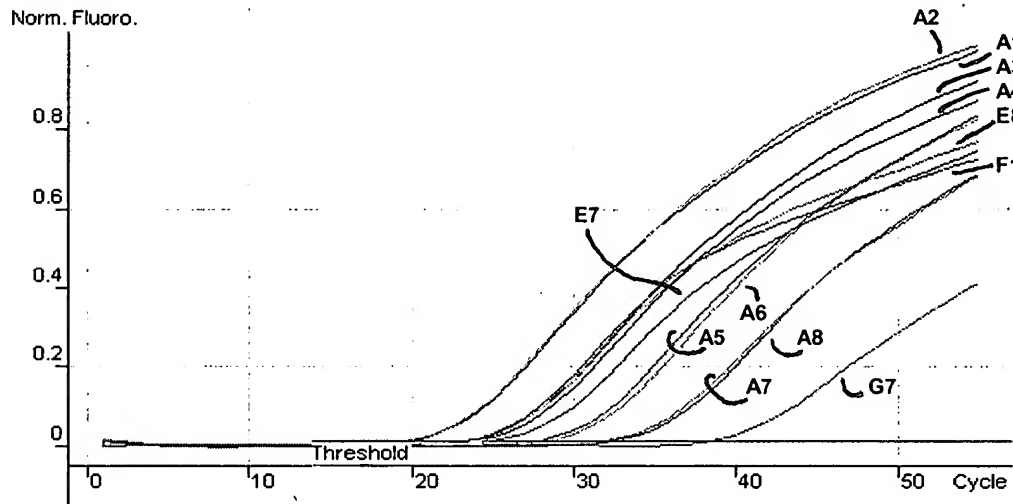
Figure 4a

REPLACEMENT SHEET

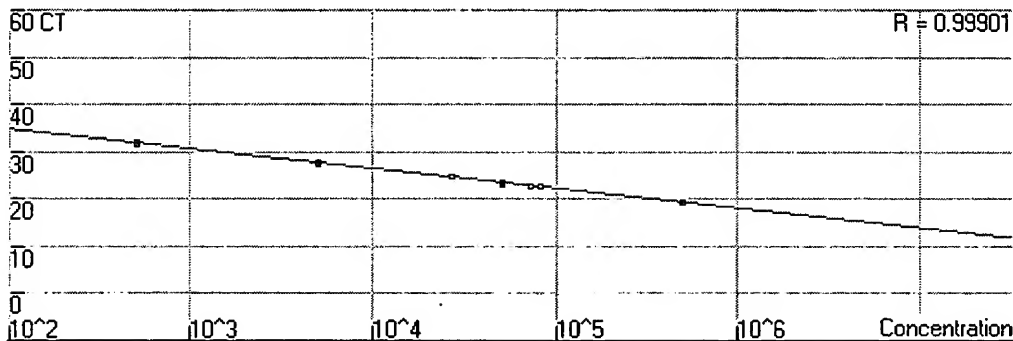
Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)
Inventor: Robert N. RICE et al.
Application No.: 10/081,646
Docket No.: 546322000100



Amplification plots and Quantitation data for Human GAPDH (Duplexed with EGFP Figure 4a)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
A1		dT/SS RNA 500ng	Standard	500,000	459,772	8.05%	19.41	0.06
A2		dT/SS RNA 500ng	Standard	500,000	491,034	1.79%	19.29	0.06
A3		dT/SS RNA 50ng	Standard	50,000	59,175	18.35%	23.15	0.21
A4		dT/SS RNA 50ng	Standard	50,000	47,005	5.99%	23.57	0.21
A5		dT/SS RNA 5ng	Standard	5,000	4,378	12.44%	27.9	0.28
A6		dT/SS RNA 5ng	Standard	5,000	5,984	19.68%	27.33	0.28
A7		dT/SS RNA 0.5ng	Standard	500	448	10.48%	32.06	0.16
A8		dT/SS RNA 0.5ng	Standard	500	530	6.10%	31.75	0.16
E7		RT Plus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		26,434		24.62	
E8		RT Plus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		81,772		22.56	
F1		RT Plus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		70,909		22.82	
G7		RT minus MM96L EGFP #22 NRO 10 ⁶ nuclei	Sample		16		38.17	

Figure 4b

REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

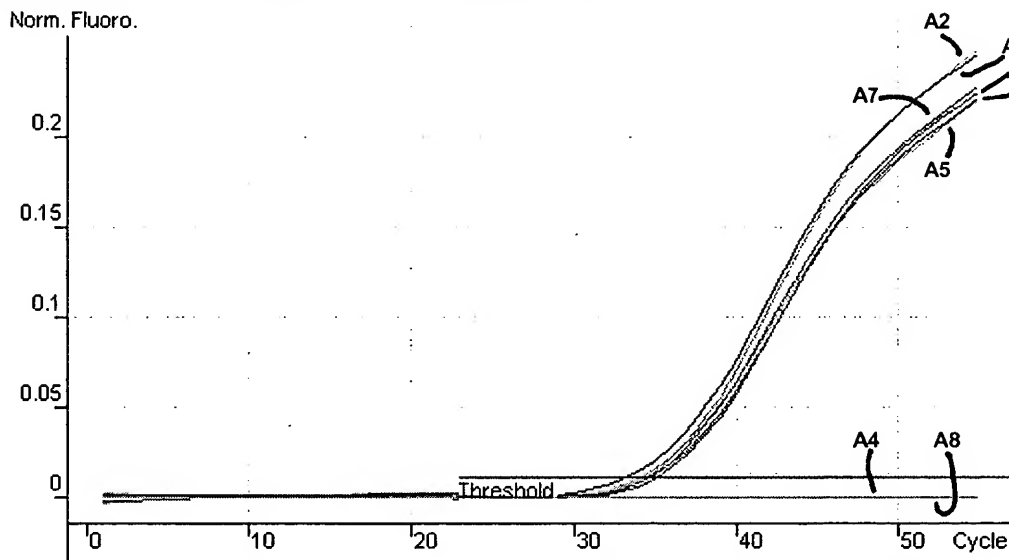
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

Docket No.: 546322000100



Amplification plots and Quantitation data for Human Endogenous HER2 (Duplexed with Human GAPDH Figure 5b)



No.	Colour	Name	Type	Ct	Ct Std. Dev.
A1	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	34.63	0.67
A2	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	34.47	
A3	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	33.4	
A4	■	NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #2.6	Sample		
A5	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	34.22	0.47
A6	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	34.74	
A7	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	35.16	
A8	■	NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #4.3	Sample		

Figure 5a

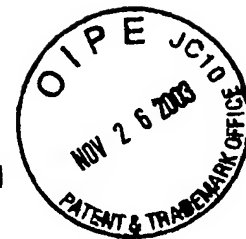
REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

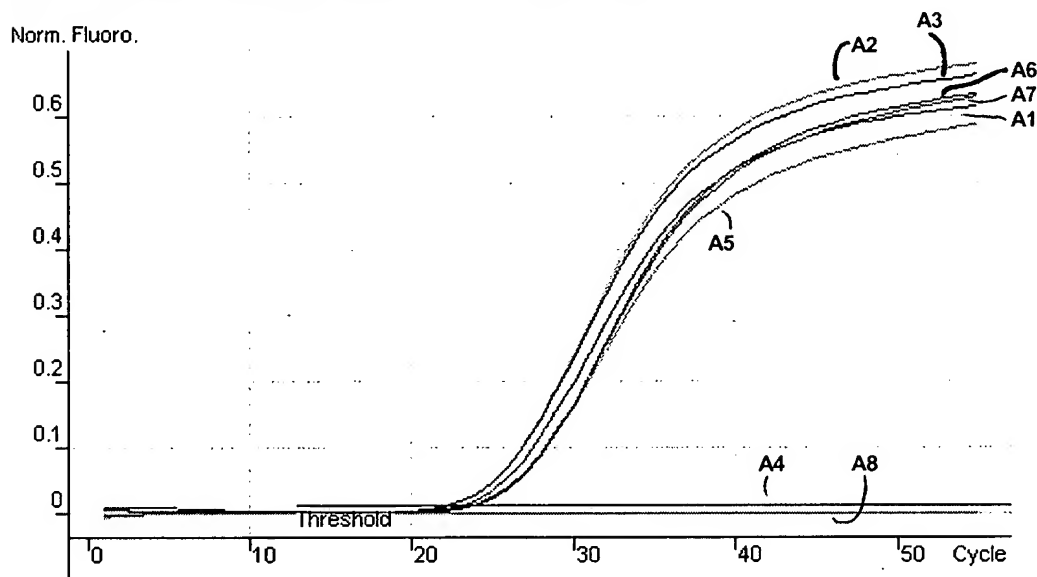
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

Docket No.: 546322000100



Amplification plots and Quantitation data for Human GAPDH (Duplexed with Human Endogenous HER2 Figure 5a)



No.	Colour	Name	Type	Ct	Ct Std. Dev.
A1	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	23.16	0.48
A2	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	22.35	
A3	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #2.6	Sample	22.31	
A4	■	NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #2.6	Sample		
A5	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	23.77	0.18
A6	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	23.71	
A7	■	NRO 10 ⁶ nuclei RT+ive MDA-MB 468 HER2 positive clone #4.3	Sample	24.05	
A8	■	NRO 10 ⁶ nuclei RTminus MDA-MB 468 HER2 positive clone #4.3	Sample		

Figure 5b

REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)

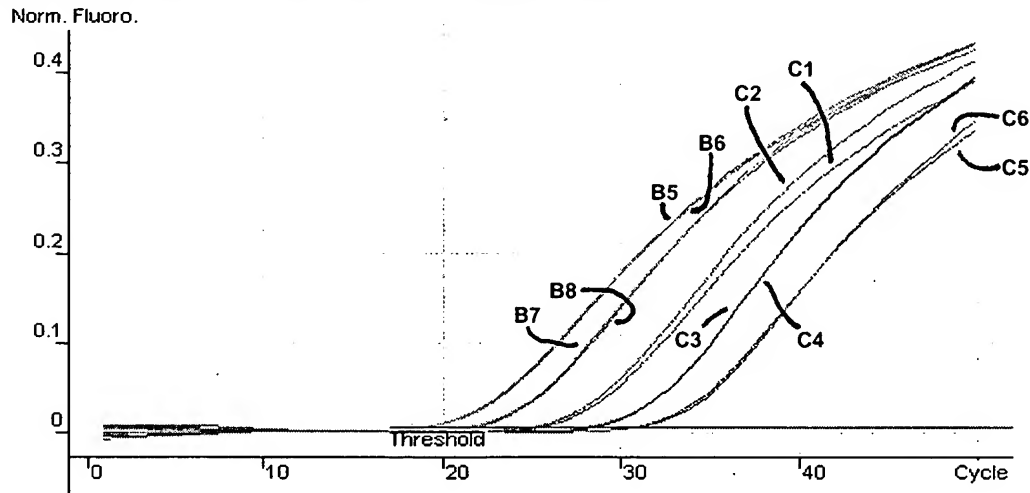
Inventor: Robert N. RICE et al.

Application No.: 10/081,646

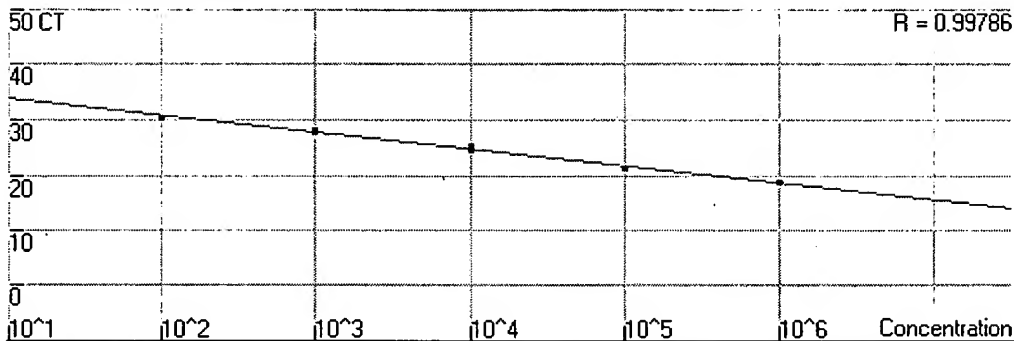
Docket No.: 546322000100



Amplification plots and Quantitation data for HER-2 Exogenous assay (Duplexed with Human GAPDH Figure 6b)



Standard Curve



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
B5		750ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000,000	955,084	4.49%	18.57	0.02
B6		750ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000,000	933,856	6.61%	18.6	0.02
B7		75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100,000	130,162	30.16%	21.23	0.10
B8		75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100,000	111,212	11.21%	21.44	0.10
C1		7.5ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	10,000	7,058	29.42%	25.12	0.34
C2		7.5ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	10,000	11,748	17.48%	24.44	0.34
C3		0.75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000	879	12.08%	27.9	0.14
C4		0.75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000	707	29.25%	28.19	0.14
C5		0.075ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100	124	24.39%	30.51	0.02
C6		0.075ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100	121	20.72%	30.55	0.02

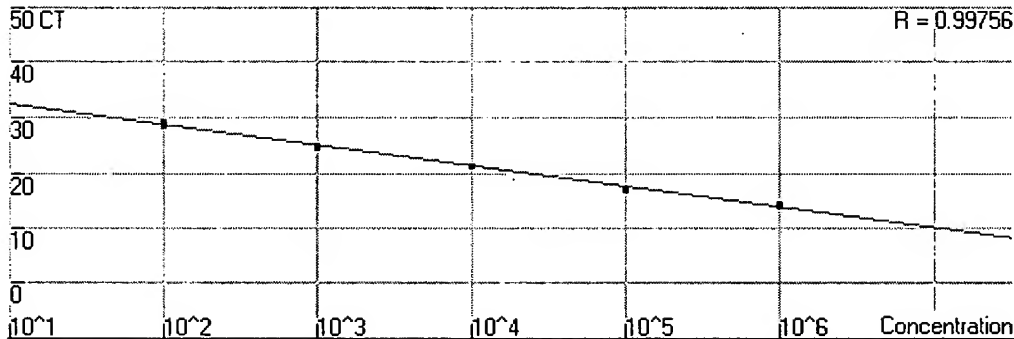
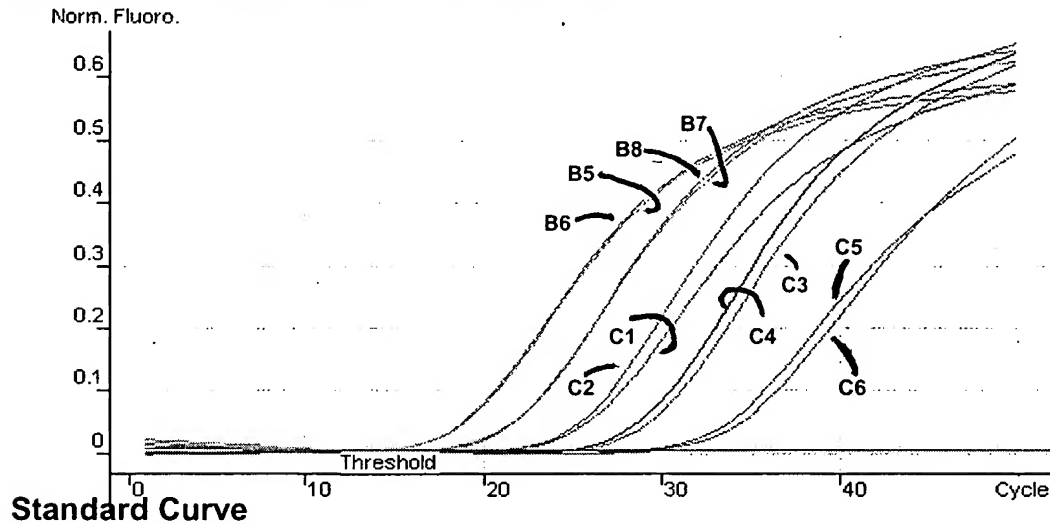
Figure 6a

REPLACEMENT SHEET

Title: Method And Kit For A Nuclear Run-On Assay
(AS AMENDED)
Inventor: Robert N. RICE et al.
Application No.: 10/081,646
Docket No.: 546322000100



Amplification plots and Quantitation data for Human GAPDH (Duplexed with HER-2 Exogenous assay Figure 6a)



No.	Colour	Name	Type	Given Conc.	Calculated Conc.	CV	Ct	Ct Std. Dev.
B5		750ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000,000	890,570	10.94%	13.95	0.21
B6		750ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000,000	687,974	31.20%	14.37	0.21
B7		75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100,000	131,712	31.71%	17.06	0.07
B8		75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100,000	120,854	20.85%	17.2	0.07
C1		7.5ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	10,000	10,472	4.72%	21.18	0.04
C2		7.5ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	10,000	9,969	0.31%	21.26	0.04
C3		0.75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000	995	0.50%	25.01	0.21
C4		0.75ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	1,000	1,296	29.59%	24.58	0.21
C5		0.075ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100	114	14.38%	28.53	0.44
C6		0.075ng DNA MDA-MB 468 4.13 HER-2 Exo/GAPDH	Standard	100	67	33.40%	29.41	0.44

Figure 6b